AMENDMENT AND REMARKS

Claims 1-87 are canceled without prejudice to their renewal in a subsequently filed application. New claims 88-137 are presented. As a result of this amendment, claims 88-137 are pending.

The following table identifies support for the new claims:

Claim	Support
88-103	original claims 1, 3, 5, 7 and 9, and 66; page 22, line 25 to page 23, line 9; page 24, line 26 to page 25, line 3; page 30, lines 6-18
104	original claim 72
105-107	original claims 10, 11 and 14
108-109	page 30, lines 5-15
110-112	original claims 15-17
113	original claim 66 and page 22, line 25 to page 23, line 1
114	original claims 5 and 7; page 24, line 26 to page 25, line 3.
115-116	original claims 5 and 9
117-118	page 30, lines 5-15
119-121	original claims 15-17
122	original claims 1, 3, 5, 7 and 9, and 66; page 22, line 25 to page 23, line 9; page 24, line 26 to page 25, line 3; page 30, lines 6-18
123-124	page 30, lines 5-15
125-127	original claims 15-17
128-130	original claims 3, 5 and 9
131-132	original claims 18 and 19
133	original claim 20; page 6, lines 8-10
134	page 22, line 25 to page 23, line 1
135-137	original claims 1, 3, 15, 52; page 8, lines 16-29; page 12, lines 10-15

Accordingly, no new matter is presented by this amendment, and the Examiner is respectfully requested to enter the amendment. The following remarks address the Examiner's comments in the order made.

I. Formal Matters

Claims 52 and 87 were objected to for informalities. This objection is rendered moot by cancellation of claims 52 and 87. New claims 103 and 116, corresponding to canceled claim 52, are grammatically correct; claim 87 is canceled without prejudice or disclaimer. Accordingly, this objection may now be withdrawn.

II. Rejections Under 35 USC § 112, first paragraph.

Claims 1, 3, 5, 7, 9-20, 23, 45, 46, 48, 50, 52-63, 66, 67, 69, 71-84, and 87 were rejected on the

basis that, while the specification is enabling for a method of detecting and isolating cells that produce a secreted protein of interest, wherein "the protein of interest comprises an antibody Fc domain; the cell surface capture molecule is an Fc receptor or a surface displayed G chimera; the detection molecule is an antibody specific for the protein of interest or, when the protein of interest is an antibody, an antigen to which the protein of interest binds; and the cell is a mammalian cell, it does not reasonably provide enablement for any secreted protein, a cell surface capture molecule that is any expressed protein, and a detection molecule is any molecule which binds to the protein of interest. Claims 1-87 are canceled without prejudice or disclaimer.

Applicant respectfully traverse this rejection as it may be applied to new claims 88-137. Applicants respectfully assert that when the instant specification is read in light of the art, one of ordinary skill in the art will understand how to practice the invention with a wide range of secreted proteins, cell surface capture molecules capable of binding the protein of interest.

The new claims are written to more clearly define the invention. New claim 88 is drawn to a method of detecting and isolating a eukaryotic cell, and further clarify that the cell surface capture molecule is capable of binding the POI, and that the POI-capture molecule complex is formed intracellularly. Complex formation occurs intracellularly, thus the intracellular environment of the ER and Golgi apparatus provides high local concentrations of each component of the complex such that high binding affinities are not required. Further, the isolated cell represents a system in equilibrium between the constantly forming intracellular complex, expression of the complex at the cell surface, dissociation of the POI from the complex and/or internalization of the complex.

New independent claim 113 differs from new claim 88 in that the eukaryotic cell produces the secreted POI, and thus the step of transfecting the cell with a nucleic acid molecule encoding a POI is eliminated.

New independent claim 122 is drawn to a more specific embodiment of the invention in which the secreted POI comprises an Fc domain and the capture molecule is capable of binding an Fc domain.

New claim 135 is drawn to a method of isolating an antibody by fusing antibody-producing cells with cells expressing a cell surface capture molecule.

Declaration Under 37 CFR § 1.132

In support of enablement of the full scope of the invention as claimed, applicants herein submit a Declaration under 37 CFR §1.132 from Dr. James P. Fandl (attached). The Declaration describes additional experiments conducted to clarify the claimed method in light of the enablement rejection.

The method of isolating cells claimed in the instant claims (for convenience termed "FASTR" for FACS-dependent autologous secretion trap) requires the construction of a host cell line that expresses a cell surface capture molecule (CSCM) capable of binding a secreted protein of interest (sPOI). The requirements of the FASTR screening system are that the CSCM be expressed at the cell surface, and there be some measurable affinity between CSCM and sPOI. Four experiments were conducted using different CSCMs-sPOIs combinations to show that the method of the invention may be used with a wide variety of capture molecules and sPOIs, both natural and synthetic, and that the method may be used to select high sPOI production host cells even with CSCM:sPOI pairs having low measurable affinity.

Example 1 examined the effect of a high or low levels of capture molecule expression on the ability of the FASTR screening to select a cell expressing a high level of a sPOI. One cell line, RGC10, contained 40 copies of the cell surface capture molecule gene (hFcγR1) and expressed at the CSCP at a high level. A second cell line, RS527, had a single copy of the same capture molecule and expressed the CSCM at a low level (western blot analysis, Fig. 1). Both cells were transfected with a plasmid expressing the same sPOI (Rc1-hFc). After selection, sorting, and expansion, mean fluorescence was plotted against sPOI production (Fig. 2) and showed that the amount of fluorescence (amount of Rc1-hFc displayed on the cell surface) and sPOI protein production were correlated. These results demonstrate that the FASTR screening method is able to identify and select a high production sPOI cell in cells expressing varying levels of the capture molecule.

A second experiment was conducted in which the extracellular domain of the Tie2 receptor (lacking the cytoplasmic TK domain) was used as the capture molecule and the sPOI was an engineered $ScFv_{C1b}$-Fc fusion protein made from a C1b monoclonal antibody specific to the extracellular domain of Tie2 receptor (Example 2). Fig. 3 shows cells stained with antibody specific for Tie2 after induction of CSCM with doxycycline (Dox). After transfection with a plasmid expressing the sPOI $ScFv_{C1b}$-Fc and conferring resistance to hygromycin, three pools of cells were selected (Fig. 4A), sorted, and expanded in cell culture. Fig. 4B shows the correlation between mean fluorescence (amount of $ScFv_{C1b}$-Fc binding to Tie2 on the cell surface) and $ScFv_{C1b}$-Fc protein production level for three selected cell bins. These results establish that the FASTR screening method can be used with capture molecules such as natural receptors in which the cytoplasmic domain is removed.

A third experiment was conducted to show that FASTR may be used with CSCM:sPOI pairs having very low affinities. In Example 3, the sPOI was a chimeric protein consisting of the Tie2 receptor-binding domain of the Tie2 ligand (angiopoietin-1 FD or FD1) fused to hFc (FD1-Fc), and the capture molecule was Tie2 lacking the cytoplasmic domain. These molecules were determined to bind with an affinity of 174 nM (BIAcore), which is considered to be a relatively low affinity (for example, the Fc-Fc receptor affinity is about 10 nM). Induced expression of the capture molecule can be detected

with an anti-Tie2 antibody or with a Tie2 ligand (Fig. 5). Pools of cells transfected with a plasmid which expresses FD1-Fc and confers hygromycin resistance were selected (Fig. 6A) and the correlation between mean fluorescence (FD1-Fc binding to cell surface-bound Tie2) and FD1-hFc protein production levels of the isolated cell pools determined (Fig. 6B). These results demonstrate that the FASTR screening method is useful for selecting and identifying high producing sPOI cells with CSCM:sPOI pairs having low affinity. Importantly, the dissociation t1/2 for FD1-Fc:Tie2 binding is less than 2 minutes, showing that any CSCM:sPOI pair with a measurable affinity can be used in the method of the invention.

The fourth experiment demonstrates that the FASTR screening method may be used with a synthetic capture molecule constructed from an antibody and a transmembrane domain which is expressed at the cell surface and displays a measurable affinity for the sPOI (Example 4). A synthetic capture molecule was constructed with the transmembrane domain of the PDGF receptor fused to an ScFv containing variable regions specific for murine kappa chain ($ScFv_{HB58}$-TM_{PDGFR}). The results demonstrate that an ScFv can be used as a functional capture molecule by targeting it to the cell membrane. Cells expressing $ScFv_{HB58}$-TM_{PDGFR} were identified by sequential incubation with the P12 antibody (specific to FD2-Fc), FD2-hFc, and FITC-conjugated anti-hIgG (Fig. 7). Fig. 8B shows that cells transfected with a plasmid encoding eYFP are stained with P12, showing that the majority of cells continue to synthesize the capture molecule. Fig. 8C shows that YFP cells mixed with different cells transfected with P12 express P12 antibody. Fig. 8D shows that FASTR was able to separate P12 positive cells from non-expressing (yellow) cells. This experiment shows that FASTR allows cells expressing a secreted antibody to be detected with the antibody's antigen.

Specific Comments Addressing the Enablement Rejection

The Examiner's allegations regarding lack of enablement will now be addressed in turn. First, the Examiner alleges that an artisan would be unable to predict the minimum affinity and dissociation rate of the cell surface capture molecule and protein of interest. Implicit in this allegation is the assumption that only molecules with particularly strong mutual affinities are suitable for use in the claimed methods. Contrary to such an assumption, the third experiment in the attached declaration shows that a cell surface capture molecule and a protein of interest having only a modest affinity (174 nM) and a short dissociation half-life (less than two minutes) are effective for use in the claimed methods. The affinity of 174 nM is less than that between an Fc domain and Fc receptor (about 5 nM) and less than that of most useful antibodies for their targets (typically 1to 100 nM (see, e.g,. Harlow and Lane (1988) Antibodies, A Laboratory Manual, at p. 28 (Cold Spring Harbor). Thus, affinities or dissociation constants required by the claimed methods are toward the lower end of those of many

paired molecules known to have mutual affinity for one another. Examples of such paired molecules include antibody and antigen (Example 2 in attached declaration) or receptor-ligand (Examples 1 and 3 in attached declaration). Alternatively, artificial molecules can be constructed or selected as illustrated by Example 4 attached to the declaration. Thus, in practice the claimed methods do not require one to identify pairs of molecules with particularly strong mutual affinities or to determine or predict some threshold level of mutual affinity at which the methods would work.

Next, the Examiner alleges additional difficulties from use of detection molecules and in particular the requirement that the detection molecule bind to some epitope of the protein of interest different from the cell surface capture molecule. The use of a detection molecule in the claimed method in binding to an epitope different from another molecule does not present greater difficulties than use of a detection molecule in conventional immunological methods. For example, it is well known to detect an antibody bound to a target using an antibody with affinity for the isotype of the target-bound antibody as the detection reagent (Harlow and Lane (1988) *supra*, at p. 591). Another well-known format is the sandwich assay in which one immobilized antibody and one solution antibody bind to a target sandwiched between them (Harlow and Lane, (1988) *supra*, at p. 579). It is agreed that the claimed methods require, as do conventional methods of immunological detection that the detection molecule bind to a different epitope on a target than is bound by another molecule. However, this would be as an apparent to the artisan as to the Examiner. "Claims need not recite... factors where one of ordinary skill...would consider them obvious." *In re Skrivan,* 166 USPQ 85 (CCPA 1970).

Next, the Examiner cites Goeddel as teaching that each cDNA or gene presents its own set of problems and there are no hard and fast rules for obtaining high-level expression. To suggest there were no general rules available for expressing proteins in 2001, the priority date of the application, is an exaggeration. Even in 1990, although Goeddel prefaces his article with the excerpt quoted by the Examiner, the bulk of the article provides generalizations to assist the reader in selecting a suitable expression system. General guidance for expression of genes is also provided by the well-known laboratory manual Sambrook et al. (2nd Ed, 1989) who devotes two entire chapters to this subject. Various well-known cell types, vectors and regulatory sequences for performing expression are even listed in undergraduate textbooks (see, e.g., Devlin (1992) Biochemistry (Wiley Liss) at pp. 790-793. Moreover, by 2001 large numbers of genes had been successfully expressed. The widespread expression of proteins in the art by 2001 indicates that such variability as exists in finding suitable conditions to express different proteins does not constitute undue experimentation to those skilled in the art. Further, the rejection appears premised at least in part on the assumption that a high level of expression is required for the claimed methods. Example 1 of the attached declaration shows that a cell surface capture molecule can capture detectable amounts of a protein of interest notwithstanding

low expression of the cell capture molecule. The example compares two cells expressing substantially different levels of the same cell surface capture molecule (FCγRF). The protein of interest was easily detectable even with the lower expressing cell.

Next, the Examiner alleges additional difficulties based on speculation that the type of lipid can influence a membrane protein's function. However, in the claimed methods, a transmembrane portion of a cell surface capture protein has the comparatively undemanding task of anchoring the protein in the membrane. By contrast, natural membrane proteins may have more complex tasks such as signal transduction or molecule transport. One would expect the simple task of providing an anchor to be less sensitive to lipid requirements than more complex tasks of natural membrane protein. Such an expectation is reinforced by Example 4 of the attached declaration. This example shows that a cell surface capture protein can be formed by linking a single-chain antibody to a transmembrane domain of a platelet derived growth factor receptor. The single-chain antibody anchored by the transmembrane domain is able to bind a protein of interest. That a transmembrane domain that is not naturally linked to a binding moiety of cell surface capture molecule can fulfill the required anchoring domain suggests that the nature of the domain and by implication its interaction with particular types of lipids are not critical to the function of a linked binding moiety.

Next, the Examiner cites Lee et al. as discussing the influence of passenger proteins on the translocation process and difficulties with protein folding in conventional cell-based display methods. However, these difficulties have not prevented obtaining conventional cellular display in many systems as illustrated by Table 1 of the reference. Moreover, the major factor underlying the alleged difficulties appears to reside in the manner of fusing the carrier protein to the passenger protein (Lee et al., p. 51, first column, first paragraph). This is not an issue in the present claims because the protein of interest is not fused to the cell surface carrier protein. Also, most of Lee's comments are directed to bacterial display systems. These comments are no longer relevant in view of the amendment of the claims to recite eukaryotic cells.

The Examiner next alleges that the teaching of the application is confined to use of two different antibody Fc binding protein and a protein of interest comprising an Fc domain. Although the application provides working examples of the noted embodiments, the application also provides broader teaching of others pairs of molecules that can be used as cell surface capture molecules and proteins of interest, for example, growth factor receptor and growth factors, and antibodies and antigens (see specification at pp. 25, 26). The attached declaration shows that such combinations are operable. Examples 2 and 4 of the declaration illustrate the claimed methods performed with antibody-binding. In Example 2, the antigen is the receptor and the antibody the protein of interest and vice versa in Example 4. Example 3 of the declaration illustrates the claimed methods using a growth factor receptor as the cell surface

capture molecule and its ligand as the protein of interest. Although the protein of interest in Examples 2 and 3 contains a linked Fc domain, this serves merely as an attachment site for a detection molecule and does not bind to the cell surface capture molecule. Moreover, Example 3 not only illustrates successful binding between a growth factor receptor and ligand pair, but provides a reasonable expectation that many pairs of molecules with detectable mutual affinities are suitable. As was discussed above, the affinities of the growth factor receptor and ligand in Example 3 are relatively low on the scale of affinities of available binding pairs.

In the aggregate, the evidence provided in the specification and the declaration shows the operability of at least five types of cell surface capture protein-protein of interest (FC receptor FC, protein G-Fc, antigen-antibody, growth factor receptor-growth factor, and antibody-antigen). The declaration also shows that the assumptions on which the rejection is, in part, premised regarding the need for a high level of expression and mutual affinity of the cell surface capture molecule and the protein of interest are wrong. When the Wands factors are reconsidered in light of the confirmation of the teaching of the application provided by the declaration, a different conclusion results. It is respectfully submitted that the artisan would have been able to select without undue experimentation a reasonable number of pairs of cell surface capture molecules and proteins of interest, and isolate or detect cells displaying the protein of interest as claimed.

In light of these remarks, applicants respectfully request that the enablement rejection be withdrawn.

III. Rejections Under 35 USC § 112, second paragraph.

Claims 1, 3, 5, 7, 9-20, 23, 45, 46, 48, 50, 52-63, 66, 67, 69, 71-84, and 87 were rejected as indefinite. This rejection is rendered moot by cancellation of the claims.

IV. Rejection Under 35 USC § 102(b).

Claims 45, 46, 48, 50, 53, 55, 58-60 and 63 were rejected as anticipated by Miltenyi et al. (1994) WO 94/091117. This rejection is rendered moot by cancellation of the claims. Miltenyi et al. describe a static system that simply binds a capture molecule to a protein of interest on the cell surface. The POI-capture molecule complex is not replaced in time because the system of Miltenyi et al. does not generate more capture molecules, and thus detection is limited to the amount of the initial exposure. In contrast, all the new independent claims specify that the cell is expressing both the POI and capture molecules such that the POI-capture molecule complex is formed intracellularly. Thus, in the FASTR system, the detected cell is in a state of dynamic equilibrium in which the sPOI and capture molecule form a complex which moves to the cell surface, which dissociates and/or is internalized.

Accordingly, in light of the above remarks and amendments, it is believed that this rejection should be withdrawn.

Conclusion

It is believed that this document is fully responsive to the OFFICE action dated 18 May 2004. In light of the above amendments and remarks, it is believed that the claims are now in condition for allowance, and such action is respectfully urged.

Fees

The Office action dated 18 May 2004 set a three-month response period to 18 August 2004. Applicants herein petition for a three month extension to 18 November 2004. The Commissioner is hereby authorized to charge Deposit Account Number 18-0650 in the amount of $980. Although it is believed that no further fees are due, in the event the Patent Office determines that fees are due, the Commissioner is hereby authorized to charge Deposit Account Number 18-0650 in the amount of those further fees.

Respectfully submitted,

Valeta Gregg, Ph.D., Reg. No. 35,127
Regeneron Pharmaceuticals, Inc.
777 Old Saw Mill River Road
Tarrytown, New York 10591
(914) 345-7400